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Genetics

Polymorphism in mitochondrial DNA of humans as revealed by restriction endonuclease analysis

(human evolution/intraspecific variation/population genetics)

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ABSTRACT **Mitochondrial DNA samples from each of 21 humans of diverse racial and geographic origin were digested with each of 18 restriction endonucleases. The sizes of the resulting DNA fragments were compared after gel electrophoresis. No differences among the samples were detected in digests with 7 of the enzymes. Analysis of digests with the remaining enzymes showed one or more differences. Each of the 21 samples could be characterized individually on the basis of these digests. All between-sample differences could be explained by single base substitutions. No evidence for sequence rearrangements (inversions, transpositions) was obtained. Fourteen of the site alterations were shared by two or more samples; six of these were shared between races. The data indicate that individuals differ from a postulated ancestral mtDNA sequence at 0.18% of their base pairs. On the basis of an estimated rate for base substitution of 1% per 10^6 years [Brown, W. M., George, M., Jr. & Wilson, A. C. (1979) *Proc. Natl. Acad. Sci. USA* 76, 1967–1971], *Homo sapiens* could have speciated or passed through a severe population constriction as recently as 180,000 years ago. The data suggest that group-specific patterns of cleavage exist. The high resolution and precision afforded by this method of analysis makes possible the investigation of many questions concerning human population genetics, evolution, and recent history.**

The mitochondrial genome of humans is a closed circular duplex DNA of ≈16,500 nucleotide pairs (1, 2). The relative positions of the mitochondrial origin of replication and ribosomal genes have been shown to be the same among vertebrate species (3–5). It is likely that the order of the remaining mitochondrial genes will also prove to be highly conserved, at least among vertebrates (2, 6). Despite this conservative feature, the base sequence of mitochondrial DNA (mtDNA) has been shown to evolve rapidly (2, 7–9). The rate of base substitution appears to be 5–10 times faster than that of single-copy nuclear DNA (2). These properties and the relative ease of its preparation make mtDNA a useful molecule to employ in studies of population genetics and evolution.

Human mtDNA samples were obtained from 21 individuals of diverse geographic and racial backgrounds (10). The fragments produced by digestion of mtDNA with 18 different restriction endonucleases have been analyzed by gel electrophoresis. The fragment patterns have been compared among the samples. An estimate for the average fraction of base pair substitutions in human mtDNA has been obtained. The amount of time elapsed since the human population was monomorphic for mtDNA has been calculated as the product of the substitution rate times this fraction. The result indicates that the human species may have passed through a severe population constriction ("bottleneck") relatively recently.

Humans were chosen for study because they are genetically outbreeding and, originally, because a large amount of mtDNA could be obtained from each individual (from placental tissue), thus enabling many analyses to be performed per sample (10). The same techniques have been successfully applied to the analysis of individual samples from much smaller animals (11, 12). The use of radioactive labeling methods make multiple analyses on even the smallest animals possible.

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MATERIALS AND METHODS

DNAs. Closed circular mtDNAs from HeLa cells (strain S3) and from human placentas were prepared as described (1, 10). The placentas were from individuals of the negroid, mongoloid, and caucasoid races, as indicated in Table 1. Bacteriophage and plasmid DNAs were prepared as described (13, 14).

Restriction Endonuclease Digestion. Abbreviations for the names of the restriction endonucleases are those used by Roberts (15). The enzymes were purchased from New England BioLabs (Beverly, MA). Digestion conditions have been described (15).

Electrophoresis of DNA. The electrophoretic analysis of DNA fragments in vertical slab gels of 1.2% and 2% agarose and of 3.5% and 5% polyacrylamide was performed as described, respectively, by Brown *et al.* (13) and by Drouin and Symons (16). Polyacrylamide gels (acrylamide-to-bisacrylamide ratio of 20:1) with dimensions of 40 × 15 × 0.08 cm were cast and run in the nondenaturing TBE buffer system described by Maniatis *et al.* (17). The *Hin*cII fragments of phage φX174 replicative form DNA (18), the *Eco*RI fragments of phage λ DNA (19), and the *Hin*dIII fragments of phage PM2 DNA (11, 20, 21) were used as size standards.

Labeling and Autoradiography of DNA. Fragments of mtDNA produced by restriction endonuclease digestion were end-labeled with ^{32}P by using the procedure of Drouin and Symons (16). Ten nanograms of mtDNA were digested to completion with a restriction endonuclease in a volume of 10 μl. One microcurie (3.7×10^4 becquerels) of an appropriate [α-^{32}P]dNTP (New England Nuclear; ≈350 mCi/mmol) was placed in a 1.5-ml snap-cap tube (Brinkmann) and dried under reduced pressure. The choice of dNTP was dictated by the base sequence of the enzymatic recognition site. The mtDNA digest was transferred to the tube containing the dried label and mixed to dissolve the label, and 0.25 unit of the large fragment (22) of *Escherichia coli* DNA polymerase I (PolI; Boehringer Mannheim) was added. The mixture was kept at room temperature (≈25°C) for 20–30 min, then 340 μl of 0.3 M sodium acetate containing 20 μg of *E. coli* tRNA (Sigma) was added and the nucleic acids were precipitated by addition of 2.5 vol of cold (−20°C) absolute ethanol and incubation at −65°C for 20–30 min. After centrifugation at 5°C for 5 min (Brinkmann, model 3514), the supernatant was decanted and the pellet was gently rinsed with 1 ml of cold 70% (vol/vol) ethanol, dried under reduced pressure, and resuspended in 5–10 μl of 10% sucrose/0.5% bromphenol blue and loaded onto a slab gel. After electrophoresis, the top support plate was removed, the gel and bottom plate were wrapped with Saran Wrap and placed, gel side down, on a sheet of Kodak X-Omat RP film for autoradiography at −70°C. Alternatively, after the top plate had been

Abbreviations: PolI, DNA polymerase I; bp, base pair(s).

Table 1. Maternal race and country of origin

mtDNA samples	Race	Birthplace
1, 6, 9, 11, 12, 16–20	Caucasoid	U.S.A.
2, 4	Caucasoid	Phillipines
13	Caucasoid	Egypt
3, 5, 10, 14	Mongoloid	China
7, 8, 15, 21	Negroid	U.S.A.

Data were obtained from medical admissions forms.

removed, filter paper (Whatman 3 MM) was placed on the gel and the filter-bound gel was dried on a heated vacuum gel drying apparatus. This latter method was preferred, because rapid and repeated exposures of the dried gel could be made, using intensifying screens (23), without the problems of warmup time, condensation, and cracking that are encountered with wet gels.

Various methods for displaying DNA bands in electrophoresis gels exist. Fluorescent methods (e.g., ethidium bromide staining) are fast and inexpensive, but require at least 3–5 ng of DNA *per band* for detection. This poses serious problems when the amount of DNA is limiting or when the detection of fragments that vary greatly in size is desired. The use of radioactive DNA coupled with the sensitivity of autoradiographic detection methods eliminates the first problem, but only certain types of *in vitro* radioactive labeling techniques also eliminate the second. These techniques attach one to a few radioactive atoms to the ends of a DNA fragment, thus making the amount of radioactivity in a gel band independent of the fragment size. Both T4 polynucleotide kinase and *E. coli* PolI are enzymes capable of end-labeling DNA fragments. T4 polynucleotide kinase labels all ends equally, but requires a DNA fragment with an extended 5′ end for efficient labeling (24, 25). This limits its usefulness, because many restriction endonucleases produce DNA fragments with blunt or 3′-extended ends. The large fragment of PolI contains a 3′-to-5′ exonucleolytic activity as well as DNA polymerizing activity (22), thus enabling it to label all three types of DNA fragment ends. PolI is active over a wide range of buffer conditions, including those used for restriction endonuclease digestion, thus requiring no buffer changes or manipulation of the DNA after digestion. The single disadvantage of PolI is that the degree of labeling among different DNA fragments may be unequal. This inequality is probably a consequence of sequence differences adjacent to the fragment ends and can make the detection of bands containing more than one fragment difficult. *Mbo* I-generated fragments gave the greatest range of intensities encountered (see Fig. 2 and ref. 16). This was not a problem with the other endonucleases employed, and increased band intensity correlated well with the presence of more than one fragment in the band.

RESULTS

Analysis of Human mtDNA After Cleavage at Hexanucleotide Sites. The patterns seen after 1.2% agarose gel electrophoresis of *Hpa* I restriction endonuclease digests (Fig. 1) were identical for 18 of the 21 samples and consisted of three bands corresponding to DNA fragments of 9900, 4290, and 2310 base pairs (bp). One sample (no. 3, Fig. 1) lacked the 9900- and 2310-bp bands and showed one new band, of 12,290 bp; two samples (no. 7, Fig. 1, and no. 15, data not shown) exhibited identical patterns in which each lacked the 9900-bp band and showed two new bands of 7590 and 1980 bp. These data suggest that the *Hpa* I site between the 9900- and 2310-bp fragments has been lost in sample 3, resulting in a fusion of these fragments. This site loss is identical to the *Hin*cII site loss reported for this sample (10), because all *Hpa* I sites are also recognized by *Hin*cII. A new site, within the 9900-bp fragment, has been gained in both sample 7 and sample 15, resulting in the loss of this fragment and the production of two new fragments of 7590 and 1980 bp. The lane containing the *Hpa* I digest of sample 10 shows, in addition to the three typically seen bands, four faint bands. These resulted from the incomplete digestion of this sample. Digestion of another portion of this sample under more exhaustive conditions resulted in complete conversion to the three typical bands (data not shown).

Analysis of the *Kpn* I-digested samples in 1.2% agarose gels showed two bands in each lane, as reported (2). However, in sample 10 the smaller fragment exhibited a decreased mobility, corresponding to a size increase of ≈80 bp. Electrophoresis of the *Kpn* I digests in 2% agarose revealed a third fragment, of ≈80 bp, in all samples except 10 (data not shown). The location of the third *Kpn* I site was not mapped, but sequencing studies (J. Drouin, personal communication) have shown it to be ≈75 bp from the *Kpn* I site at 4 map units (2).

The patterns produced by electrophoresis of the samples after sequential digestion with *Pvu* II, *Sac* I and *Xho* I were identical for all 21 samples. Human mtDNA has a single site for each of *Pvu* II and *Xho* I and two sites for *Sac* I. The locations of these sites in human mtDNA are known (2).

Digests of the samples with the restriction endonucleases *Bam*HI, *Eco*RI, *Hin*cII, *Hin*dIII, *Pst* I and *Xba* I, discussed in detail elsewhere (10), showed only three atypical patterns: an identical *Bam*HI site gain in samples 2 and 9, and a *Hin*cII site loss in sample 3 (10). The remainder of the samples showed only the patterns typical for these enzymes.

Analysis of Human mtDNAs After Cleavage at Tetranucleotide Sites. The mtDNA fragments produced by digestion with the enzymes *Alu* I, *Hae* III, *Hin*fI, *Hpa* II, *Mbo* I, and *Taq* I were end-labeled with ^{32}P and analyzed by electrophoresis in 40-cm-long 3.5% and 5% polyacrylamide gels. The positions of the fragments in the gels were detected autoradiographically. Drouin and Symons (16) have reported that *Mbo* I digestion produces 23 fragments, which migrate as 21 distinct bands. Due to a high background of radioactivity in the size range below ≈30 bp when [α-^{32}P]dGTP was used as label, the smallest *Mbo* I fragment observed was the 33-bp fragment (fragment 22 in ref. 16). Typical *Mbo* I patterns were exhibited by 13 of the 21 samples. These are represented by nos. 3, 6, and 7 in Fig. 2. Atypical patterns were seen in samples 1, 2, 4, 5, 8, 9, 10, and 15 (Fig. 2). On the basis of fragment size, the atypical patterns can be explained either by the loss of a typically occurring *Mbo* I site or by the generation of a new site where there is typically none. The atypical *Mbo* I patterns exhibited by samples 2 and 9 are identical to each other, as are those for samples 4 and 8. The site gain seen in samples 2 and 9 corresponds to the *Bam*HI

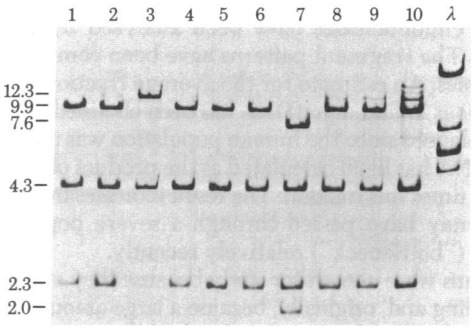

FIG. 1. A 1.2% agarose gel electrophoresis of *Hpa* I digests of human mtDNA samples 1–10. The band patterns of samples 3 and 7 differ from those of the other samples. The DNA fragment sizes, shown on the left in bp × 10^{-3}, were estimated from a calibration curve using *Eco*RI-digested λ DNA (above) and *Hin*dIII-digested PM2 DNA (not shown) as size standards. Faint bands are due to incomplete digestion.

site gain previously reported for these samples (10), because the *Mbo* I recognition sequence (G-A-T-C) is contained within that for *Bam*HI (G-G-A-T-C-C). This is confirmed by a comparison of the locations of these sites in the human mtDNA cleavage maps for *Bam*HI (10) and for *Mbo* I (16, 26). The pattern exhibited by sample 5, Fig. 2, is probably due to loss of the *Mbo* I site between the fragments contained in the bands corresponding to fragments of 780 and 25 bp, thus indicating that these fragments are adjacent. The loss of the 25-bp fragment in this sample could not be confirmed, due to the high background of radioactivity in this size region of the gel, mentioned earlier. This result contradicts the published *Mbo* I map (16, 26), in which the 780- and 25-bp fragments are shown as nonadjacent. Unpublished sequence data confirm that the placement of the 25-bp fragment in the map as published is incorrect (J. Drouin, personal communication). The adjacent location of the 780- and 25-bp fragments, suggested by the analysis of sample 5, remains to be confirmed.

The intensity of different bands within a sample lane can be seen to vary over a large range (e.g., compare the bands at 620 and 600 bp in Fig. 2). This variation is highly reproducible and is an artifact of the labeling method (see *Materials and Methods*); it is not due to incomplete digestion, because the pattern is unaffected by more exhaustive digestion and because the sum of the fragment sizes is equal to one mitochondrial genome.

Digestion with *Hin*fI typically produced 32 fragments, which migrated as 31 electrophoretically distinct bands (Fig. 3). The corresponding fragment sizes account for 99% of the mitochondrial genome. Ten of the samples (1–3, 6, 9, 11–13, 16, and 19) showed identical patterns. Eleven of the samples exhibited atypical patterns; all can be explained by single-site losses or gains. Two, 7 and 15, showed identical patterns, caused by the gain of a *Hin*fI site in the 385-bp fragment that resulted in the production of the 360- and 25-bp fragments observed. Seven samples (5, 7, 10, 14, 15, 17, and 21) exhibited a loss of the 875-bp fragment and appearance of a second 890-bp fragment, presumably the result of fusion of the 875-bp fragment with a postulated adjacent 15-bp fragment.

In addition to this kind of atypical *Hin*fI pattern, however, two of the atypical samples (7 and 15) and three typical samples (8, 13, and 19) showed a second kind of pattern. This consisted of the appearance of faint atypical bands that could not be rationalized simply by a site gain or loss. These bands were also characterized by their relative lack of radioactivity, because overexposure of the autoradiogram was necessary for their visualization. Such bands, faintly visible in Fig. 3, could reflect within-sample heterogeneity. The presence of "substoichiometric" bands in *Hae* III digests of mtDNA has been reported (9). However, upon further digestion the intensity of these bands decreased dramatically. A possible explanation is that these bands represent incompletely digested fragments containing *Hin*fI sites that are cleaved more slowly than usual. Tenfold differences in the kinetics of site cleavage are known (19). In the present study this phenomenon was observed only in digests with *Hin*fI.

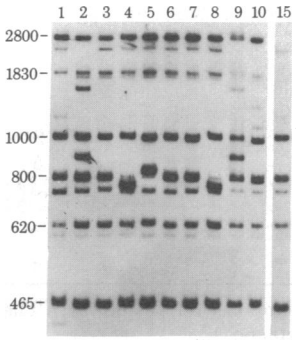

FIG. 2. A 3.5% polyacrylamide gel of *Mbo* I digests of human mtDNA samples 1–10 and 15. All atypical fragments observed were ≥450 bp, hence the portion of the gel containing smaller fragments is not shown. The band pattern shown by samples 3, 6, and 7 was typical for the majority of the 21 samples analyzed. Samples 2 and 9 show identical atypical patterns, as do samples 4 and 8. The DNA fragment sizes, in bp, are shown on the left. The faint bands are completely digested fragments that, because of the labeling method, do not label well.

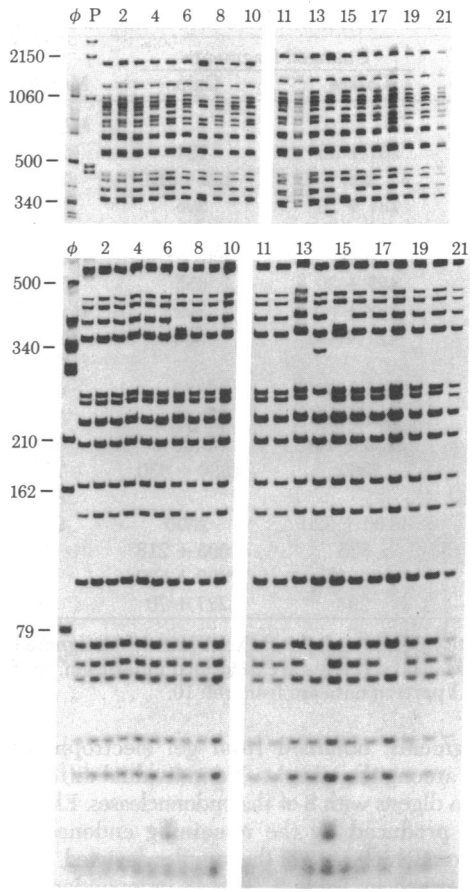

FIG. 3. Polyacrylamide gel electrophoresis of *Hin*fI digests of human mtDNA samples 1–21. Upper gel, 3%; lower gel, 5%. The typical pattern of bands is shown by samples 1–3, 6, 9, 11–13, and 19. Among the atypical patterns, an example of a shared morph may be easily seen in samples 7 and 15, in which the typical band at the position corresponding to 390 bp has been lost (both gels) and two atypical bands occur at positions corresponding to 373 and 27 bp (lower gel). The absence of the 66-bp fragment in samples 14 and 18 (lower gel) results, in both, from a site loss. This is not a shared morph, however, because the lost site is at one end of the 66-bp fragment in sample 14 and at the other end in sample 18 (see Table 2). Fragment sizes, given on the left, were estimated by using *Hin*cII digests of ϕX174 replicative form DNA (ϕ) and *Hin*dIII digests of PM2 DNA (P, upper gel) as size standards.

The numbers of fragments typically produced by digestion with the remaining enzymes were: *Alu* I, 48; *Hae* III, 46; *Hha* I, 17 (10); *Hpa* II, 23 (10); *Taq* I, 28. Fragments of <30 bp were not scored. The sums of the fragment sizes were equal to 100 ±3% of the mtDNA for digests with all enzymes except *Alu* I, which summed to 94%. This lower value is probably due to bands containing more than one fragment not being scored as such. Reexamination of the *Hpa* II digests resulted in the observation, in sample 13, of an atypical site gain not reported in ref. 10. All atypical patterns observed could be explained by site gains or losses, as summarized in Table 2.

DISCUSSION

Human mtDNA Exhibits Sequence Polymorphism. Mitochondrial DNA samples from each of 21 humans of diverse racial and geographic origin were digested with each of 18 restriction endonucleases. Estimates of the sizes of the resulting

Table 2. Tetranucleotide sites in human mtDNA exhibiting cleavage polymorphism for seven restriction endonucleases

Morph	Changed fragments: Losses	Changed fragments: Gains	Morph	Changed fragments: Losses	Changed fragments: Gains	Morph	Changed fragments: Losses	Changed fragments: Gains
Alu I			*Mbo* I			*Hin*fI		
2	157 + 30	184	2	1650	1100 + 450	2	810	795 + [15]
3	235	222 + [13]	3	2400	1480 + 880	3	875 + 15	890
4	181 + 26	205	4	780	750 + [30]	4	390	367 + 28
5	417 + 184	600	5	780 + [30]	810	5	1050 + 810	1750
6	388	200 + 160	6	2400	1300 + 1070	6	1050 + 760	1685
7	427 + 30	455	7	≈1800	1200 + 550	7	256 + 66	324
8	388 + [4]	392	*Hae* III			8	630 + 66	695
9	427 + 172	600	2	140	133 + [7]	9	256 + [4]	260
Hha I*			3	375 + 61	436	10	256	250 + [6]
2	2260 + 286	2480	4	565	505 + 60	*Taq* I		
3	286 + 180	458	5	1140	800 + 335	2	815 + 81	910
4	1230 + 180	1420	6	153 + 32	180	3	510	390 + 106
5	2260	1630 + 630	7	375 + 140	520	4	310 + 147	475
Hpa II			8	236	255 + [11]	5	2900	1900 + 1080
2	2100 + 520	2600	9	750 + 565	1340	6	895 + 111	1020
3	835	605 + 218				7	269	163 + 90
4	2500	2000 + 520						
5	298	227 + 70						

The most typical morph for each enzyme was designated morph no. 1. Changes are with reference to the pattern typical for each enzyme (i.e., morph no. 1). All fragments are described in terms of bp. Sizes of fragments postulated, but not observed, are in brackets.
* The *Hha* I pattern data are from ref. 10.

DNA fragments, obtained from gel electrophoresis, were compared among the samples. No individual differences were observed in digests with 8 of the endonucleases. Electrophoresis of digests produced by the remaining endonucleases gave patterns from which 1–12 of the samples deviated. These results are summarized in Table 3. The 48 independent alterations observed include 22 site gains and 26 site losses. No heterogeneity was observed within the mtDNA samples. Possible within-sample heterogeneity has been reported (9, 27), but no convincing demonstration of this exists. The estimated sensitivity of the autoradiographic method employed is sufficient for the detection of a site difference held in common by 2% of the molecules within a sample. Despite this, a large amount of heterogeneity could go undetected. For an endonuclease with R sites, R mtDNA subpopulations could exist, each lacking a different typical site and each comprising an undetectable fraction, n_i, of the total (i goes from 1 to R). The fraction, F, that contained atypical site losses would be $R\Sigma n_i$. For example, for *Hae* III, assuming an average n_i of 0.5%, F = 23%.

The average amount of nucleotide change in human mtDNA, expressed as substitutions per base pair, can be estimated by the method of cleavage site comparisons (28, 29). Using only the data from analysis of digests with enzymes recognizing tetranucleotide sites (E through K, Table 3), an average of 217 sites in each sample (equivalent to 868 bp, or 5.3% of the mtDNA) were compared. An average value of 0.0018 base substitutions per bp *per individual* was obtained. The average diversity observed between as compared to within racial groups did not differ. The *pairwise* nucleotide diversity observed in humans, 0.0036 substitutions per bp, is significantly below that reported for rodents (12, 30) or observed in apes (unpublished).

The atypical patterns observed can all be explained by either the loss or gain of a site due to a single base substitution event. An alternative, the addition or deletion of one or a few bases within a site, seems a less likely explanation. Individual differences in methylation are another possible source of variation. An elegant study of the molecular basis for potentially heritable pattern variation in eukaryotic DNA methylation has been published (31, 32). However, in animals this type of variation should affect only those enzymes with the dinucleotide C-G in their recognition sites (e.g., *Hha* I, *Hpa* II, and *Taq* I) and should not affect those that lack it (e.g., *Alu* I, *Hae* III, *Hin*fI, and *Mbo* I). Because the observed variation was greater with this latter group of enzymes, and for the additional reasons discussed by Brown and Goodman (10), it seems unlikely that differences in methylation contribute significantly to the variation seen. Finally, due to the small amount of variation

Table 3. Distribution of restriction endonuclease polymorphisms in mtDNAs from 21 humans

Endonuclease	Morph in sample: 1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
A. *Bam*HI		2							2												
B. *Hpa* I			2				3								3						
C. *Kpn* I										2											
D. *Hin*cII			2																		
E. *Alu* I	2			3, 4	5, 6	2	7	3		6	2		2		7, 8			9	2		
F. *Hae* III				2	3			2		3	3	3, 4		5		4	6, 7	3		8	9
G. *Hha* I	2		3						3		4										5
H. *Hin*fI				2	3		3, 4, 5	2		3				3, 6, 7	3, 4		3	8		9	3, 10
I. *Hpa* II		2, 3					4						5								
J. *Mbo* I	2	3		4	5			4	3	6					7						
K. *Taq* I							2, 3							4	5				6		7

The samples were monomorphic for the following enzymes: *Eco*RI, *Hin*dIII, *Xba* I, *Pst* I, *Pvu* II, *Sac* I, and *Xho* I (see ref. 10 and text). The characteristics of the numbered polymorphisms are as explained in Table 2 and the text. A blank indicates morph no. 1.

Table 4. Polymorphisms shared by multiple samples

Samples	Morphs shared*		Race†
	Site gains	Site losses	
4, 8	E-3, F-2, H-2, J-4	—	C, N
7, 15	B-3, H-4	E-7, H-3	N
5, 10	E-6	F-3, H-3	M
12, 16	F-4	—	C
2, 9	J-3	—	C
1, 6, 11, 13, 19	—	E-2	C
11, 12, 18	—	F-3	C
14, 17, 21	—	H-3	C, M, N
3, 9	—	G-3	M, C

* The morphs are as designated in Table 2.
† C, caucasoid; M, mongoloid; N, negroid; cf. Table 1.

observed, it is unlikely that more than one base substitution per site has occurred.

Sequence Heterogeneity May Suggest a Recent Population Constriction. The rate of base substitution of mammalian mtDNA has been estimated to be 0.5–1.0% per lineage per 10^6 years (2). At this rate, the amount of sequence heterogeneity observed, 0.18%, could have been generated from a single mating pair that existed 180–360 $\times$ 10^3 years ago, suggesting the possibility that present-day humans evolved from a small mitochondrially monomorphic population that existed at that time. The basic similarity in the cleavage patterns of the samples indicates that the major racial divergences have occurred since then.

Shared Polymorphisms May Indicate Group Affinities. As noted, many of the site alterations observed are shared by two or more samples. These shared morphs, summarized in Table 4, could have arisen either by inheritance from a common ancestor or by independent parallel acquisition. Parallel acquisition seems unlikely, given the low level of variation present. However, in the case of shared site losses it cannot be ruled out, because the same site could be lost in different lineages due to substitutions at different bases within the site. The most striking shared morph is, perhaps, the site loss, H-3, that occurs in 7 of the samples (Tables 3 and 4). These samples represent all three races, but not on a proportionate basis. Only 1 caucasoid sample is represented, out of 13 total, whereas 3 of 4 negroid and 3 of 4 mongoloid samples show the loss. This site loss may represent a relatively ancient variant that was present in most human populations before the occurrence of racial divergence.

Several of the atypical morphs are seen to occur only intraracially (Table 4). Because of their lower probability of occurrence, shared site gains are a more significant indicator of genetic relationships than are shared site losses. By this criterion a number of racial affinities are seen. However, the most closely related samples analyzed are 4 and 8, which share 4 atypical site gains. These samples show no affinity with any of the other samples and differ from each other only by the presence, in 4, of a site loss, E-4. Sample 4 is identified as a Phillipine-born caucasoid and 8 as a U.S.A.-born negroid. Unfortunately, no further information on the samples has become available.

Although these data do not unequivocally demonstrate the existence of group-specific mtDNA restriction endonuclease fragment patterns, they do suggest this as a strong possibility. Both larger sample sizes and more extensive genealogical information are needed for an accurate assessment of this. The high degree of precision afforded by these analyses indicates that it will be possible to obtain data that may help answer many of the questions about human population genetics, prehistory, and recent evolution.

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